OBSERVATIONS ON THE KINETICS OF THE CYSTINE CATALYSIS OF THE IODINE–AZIDE REACTION

by

SØREN LØVTRUP[1]

1. INTRODUCTION

The iodine-azide reaction

$$2\,N^-_3 + I_2 \rightarrow 3\,N_2 + 2\,I^-$$

which is catalyzed by various organic and inorganic sulfur compounds was described by RASCHIG (8) in 1915. Since then it has been paid relatively little attention, except from a mere qualitative point of view, see f. inst. FEIGL (3), who uses this reaction for identification of sulfides, thiosulfates and thiocyanates. The kinetics of the reaction remained obscure until recently, when HOFMAN-BANG (5) started to investigate this problem. He has worked especially with carbon disulfide and the polythionates (trithionate, tetrathionate etc.) as catalysts. His results with carbon disulfide, which are now being published, will be dealt with in the discussion.

The work on the iodine-azide reaction in this laboratory was started with the aim to use the reaction in connection with the Cartesian diver (HOLTER and LINDERSTRØM-LANG (6)) as a tool for the microdetermination of some compounds catalyzing the reaction (see HOLTER and LØVTRUP (7)). Among these catalysts we had our interest focussed on the organic sulfhydryl compound, cysteine and its corresponding disulfide, cystine.

These compounds had previously been investigated by FRIEDMAN (4) who did some quantitative experiments on the iodine-azide reaction catalyzed by various organic *–SH* and *–SS*-compounds. Measuring the N_2^- evolution in the BARCROFT differential manometer he could ascertain

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that sulfhydryl compounds (e. g. cysteine and glutathione) catalyzed the reaction at a much greater rate than the corresponding disulfides. But on the other hand he found that it was impossible to measure the actual catalysis rate of the –SH-compounds, since they were oxidized by the iodine to disulfides within ten minutes.

Before we could start the experiments in the diver we had to know some aspects of the kinetics of the reaction. In the paper mentioned above, FRIEDMAN had concluded that the iodine-azide reaction was a coupled oxidation, i. e. coupled with a reversible oxidation of sulfhydryl to disulfide. This conclusion was not very convincing, and scarcely warranted by the nature of his experiments. As the results of HOFMAN-BANG were in complete disagreement with FRIEDMAN's theory, we found it necessary to reinvestigate the cystine catalysis of the iodine-azide reaction. The preliminary experiments showed that the cystine reaction differed in certain ways from the results which HOFMAN-BANG had obtained with other catalysts, and we have therefore investigated the kinetics of the cystine reaction somewhat further.

2. EXPERIMENTAL

2.1. Method and Materials.

The reaction was followed by measuring the N-evolution in the WARBURG apparatus. Temperature: 25.0 °C. In the main chamber of the vessel were pipetted 1 ml azide solution and 1 ml iodine-potassium iodide solution; the side tube contained 1 ml cystine solution. The mixing was done after 30 minutes incubation in the thermostat. In the preliminary experiments cystine was dissolved in glycine buffer of pH 3.1 (2.5×10^{-4} *M*). When 1 ml of this solution was mixed with 1 ml 0.4 *M* azide and 1 ml 0.05 *M* I_2, in 0.5 M *KI* the resulting pH was 7.1 owing to the buffer capacity of the azide-azoimide system. After 20 minutes of reaction pH had dropped to 6.1. The effect of this change ought to be rather small since the iodine-azide reaction is proportional to the free azide ion concentration (HOFMAN-BANG (5)), which is not significantly altered by the shift in pH.

In the later experiments azide concentration was increased considerably, and to obtain a better control of pH and at the same time avoid the very high concentration of buffer necessary in this

case, it was decided to use the system N^-_3 –N_3H as buffer. The experiments were carried out in a medium, where the ratio azoimideazide was 1.0. The pH of a reaction mixture was before addition of cystine 4.45 and after the N_2–evolution had ceased 4.41. When concentration of azide is mentioned in these experiments, it always means concentration of N^-_3. Cystine was dissolved as cystine hydrochloride to a 2.5 $\times 10^{-4}$ *M* stock solution. The sodium azide was a pure preparation from SCHERING, the iodine from MAY and BAKER, resublimated. The cystine had been prepared in the chemical department of the Carlsberg laboratories. The other chemicals used (*KI, KCl, NaCl*) were commercial pure preparations.

2.2. Preliminary Experiments.

It was shown clearly by our first experiments that the rate of N_2–evolution is directly proportional to azide concentration. This may be seen from table 1.

Table 1.

Influence of azide on the rate of N_2-evolution.

Relative azide conc.	8	4	2	1
N_2-evolution in 20 minutes (Moles $\times$ 10^{-6})	11.62	5.80	2.78	1.39
N_2-evolution/azide conc.	1.45	1.45	1.39	1.39

The relative azide concentration 8 corresponds to the addition of 1 ml 0.40 *M* sodium azide. 1 ml 2.5 X10^{-4} cystine and 1 ml 0.05 *M* I_2 in 0.5 *M KI* were added to each vessel.

When the cystine concentration was varied a similar result was obtained (table 2).

Table 2.

Influence of cystine on the rate of N_2-evolution.

Relative cystine conc.	8	6	4	2
N_2-evolution in 20 minutes (Moles $\times$ 10^{-6})	11.25	8.16	5.35	2.70
N_2-evolution/azide conc.	1.41	1.36	1.34	1.35

The relative cystine concentration 8 corresponds to 1 ml 2.5 $\times$ 10^{-4} *M* cystine. 1 ml 0.05 *M* I_2 in 0.5 *M KI* and 1 ml 0.40 *M* sodium azide were added.

Variation of the iodine concentration showed that although the rate of N2–evolution increased with increasing iodine concentration, the yield of N_2 per mole of iodine decreased definitely with higher iodine concentration (table 3).

Table 3.

Influence of iodine on the rate of N_2-evolution.

Relative iodine conc.	8	4	2	1
N_2-evolution in 20 minutes (Moles × 10^{-6})	11.68	6.60	3.35	2.04
N_2-evolution/iodine conc.	1.46	1.65	1.78	2.04

The relative iodine concentration 8 corresponds to 1 ml 0.05 *M* I_2 in 0.5 *M KI*. The iodide concentration was the same in all cases. 1 ml 0.40 *M* azide and 1 ml 2.5 × 10^{-4} *M* cystine were added.

Increasing iodine concentration thus failed to increase the rate of N2–evolution in the same ratio. This is best explained by the assumption that the rate actually is proportional to the iodine concentration, but that the iodine at the same time has another effect, viz. destruction of the catalyst. This agrees well with the results of SHINOHARA (9), who showed that cystine is oxidized to cysteic acid according to the stoichiometric equation

$$RSSR + 5\ I_2 + 6\ H_2O = 2\ RSO_3H + 10\ HI,$$

where *RSSR* represents cystine.

It therefore seemed desirable in this investigation to change the concentrations of the components of the reaction system in such a way that all cystine was oxidized during the experiment. A series of experiments were done where the ratio iodide: cystine was increased about 50 times. These experiments will be described in the following two sections.

2.3. The Cystine Oxidation.

In the experiments to be reported here, the cystine oxidation could be followed by the change in the rate of N_2–evolution, because the concentrations of iodine, azide, (and iodide) were so large, that the changes occuring during the reaction were negligible. Since the only varying factor was cystine concentration, the variation of the N_2–evolution must be due to this cause. The N_2–evolution was found to be a first order reaction; if $\log \frac{\Delta N_{2(\infty)} - \Delta N_{2(t)}}{\Delta N_{2(\infty)}}$ was plotted as ordinate and time t as abscissa, a straight line was obtained. The slope of this curve was proportional to iodine concentration. We thus have:

$$\frac{\Delta N_{2(\infty)} - \Delta N_{2(f)}}{\Delta N_{2(\infty)}} = e^{-k[I_2]-t} \qquad \textbf{(1)}$$

where $\Delta N_{2(\infty)}$ represents N_2–evolution at the time when all cystine has been oxidized and $\Delta N_{2(t)}$ the N_2–evolution at the time t.

That the constant k is the velocity constant of the cystine oxidation may be seen from the following consideration: Differentiation of (1) with respect to t gives

$$\frac{d\Delta N_{2(t)}}{dt} = \Delta N_{2(\infty)} \cdot k \cdot [I_2] \cdot e^{-k[I_2] \cdot t}, \qquad (2)$$

But $\frac{d\Delta N_{2(t)}}{dt}$, the rate of N_2–evolution at the time t is proportional to the cystine concentration at the same time, $[RSSR]_t$, so that we may write:

$$[RSSR]_t = A \cdot \Delta N_{2(\infty)} \cdot k \cdot [I_2] \cdot e^{-k[I_2] \cdot t} \qquad (3)$$

where A is a proportionality factor. It follows from (3), that $A \cdot \Delta N_{2(\infty)} \cdot k\,[I_2]$ equals cystine concentration at zero time, thus

$$[RSSR]_t = [RSSR]_0 \cdot e^{-k[I_2] \cdot t} \qquad (4)$$

The observed values of k are listed in table 4.

2.4. The Iodine-Azide Reaction.

It was found in the preliminary experiments (tables 1, 2 and 3) that the rate of N_2–evolution was proportional to cystine and azide concentration, while the dependence on the iodine concentration was somewhat more complicated. If we assume that there is also a direct proportionality to iodine concentration, and that the deviations (table 3) are due to cystine oxidation, we obtain the expression:

$$\frac{d\,\Delta N_{2(t)}}{dt} = k_1\,[RSSR]_t \cdot [N_3^-]\,[I_2]. \qquad (5)$$

If we insert (4) into (5) we have

$$\frac{d\,\Delta N_{2(t)}}{dt} = k_1 \cdot [RSSR]_0 \cdot [N_3^-]\,[I_2] \cdot e^{-k \cdot [I_2] \cdot t}. \qquad (6)$$

Integration of (6) between the limits $t = 0$ and $t = \infty$ gives us

$$\Delta N_{2(\infty)} = \frac{k_1}{k}\,[RSSR]_0\,[N_3^-] = C\,[RSSR]_0\,[N_3^-]. \qquad (7)$$

We thus arrive at the result that the total N_2–formation is independent of the iodine concentration and directly proportional to the azide concentration and the cystine concentration at zero time. The values of C, calculated from the results obtained are listed in table 4. The constancy of these values thus supports the above assumption.

Table 4.

Iodine conc. mol/l	Azide conc. mol/l	Cystine conc. ($t = 0$) (mol/l) $\times 10^{-6}$	k l/mol $\times$ min	C l²/mol
0.0250	0.167	6.5	0.128	11.5
0.0333	0.167	5.2	0.129	11.8
0.0333	0.167	6.5	0.126	11.2
0.0333	0.167	7.8	0.125	11.4
0.0417	0.167	6.5	0.128	11.7
0.0500	0.125 [1]	6.5	0.129	11.2
0.0500	0.083 [1]	6.5	0.131	11.6

[1] N_3^- replaced by Cl^-.

The values of the constants k and C correspond to definite concentrations of iodine and total salt, resp. 0.333 and 0.667 M, and to a definite pH: 4.4. Some few experiments have been done where these factors were altered, but our results are too few to make any quantitative statements about the influence of these variables. It will be sufficient to mention that we have found repeatedly that iodide inhibits both cystine oxidation and N_2-formation.

Some few experiments were done to investigate the effect of incubating azide and cystine for a long time before the reaction was started. These experiments were performed under conditions similar to the preliminary experiments. The N_2–evolution after 20 minutes of reaction was found to be 4.40×10^{-6} moles when azide and cystine were incubated for 20 hours, while the controls gave 4.75×10^{-6} moles. This result will be referred to in the discussion.

3. DISCUSSION

Before we discuss our own results it is necessary to mention the main results of HOFMAN-BANG (5). The rate of reaction (N_2–formation) has been found to be proportional to azide and catalyst concentration, but independent of iodine concentration, when the catalyst was carbon disulfide or a polythionate. From the work of BROWNE and HOEL (1) it is known that azide and carbon disulfide form a complex, the azide dithiocarbonate ion, which reacts with iodine under N_2–evolution. HOFMAN-BANG has shown that CS_2 is re-formed and reacts again with azide:

$$2CS_2 + 2N_3^- \underset{\text{II}}{\overset{\text{I}}{\rightleftharpoons}} 2SCSN_3^- \xrightarrow[\text{III}]{I_2} 2I^- + (SCSN_3)_2 \xrightarrow[\text{IV}]{2N_3^-} 2CS_2 + 3N_2 + 2N_3^-.$$

To explain the found dependence on the concentrations of the reactants it has been assumed that the rate-determining step

in the sequence of reactions is the formation of the azide-carbondisulfide complex, while the following reactions with iodine leading to N_2–evolution are instantaneous.

If the reaction with cystine as a catalyst proceeds in an analogous manner, we can illustrate it in the following way:

$$RSSR + N_3^- \underset{\text{II}}{\overset{\text{I}}{\rightleftharpoons}} RSSRN_3^- \xrightarrow[\text{III}]{I_2} I^- + RSSRN_3I \xrightarrow[\text{IV}]{N_3^-} I + RSSR + 3N_2$$

where the rate of IV is assumed to be very great in comparison to the rates of the other reactions. The question is now whether this analogy can be upheld and the formation of a cystine-azide complex assumed in spite of the difference between our results and those of HOFMAN-BANG. The latter demonstrated the rate of N_2–evolution to be independent of iodine concentration, while we found direct proportionality. The answer can be obtained if we employ the theory of chain reactions, put forward by J. A. CHRISTIANSEN (2). According to this we may form the following expression for the above sequence of reactions

$$\frac{1}{s} = \frac{1}{k_I [RSSR] [N_3^-]} \left(1 + \frac{k_{II}}{k_{III} [I_2]}\right)$$

s is the rate of reaction, and k_I, k_{II} etc. are the velocity constants of reactions I, II etc. The differences between the results with carbon disulfide and cystine may be explained by differences in the rates of the reactions II and III in the two cases. If in the case of CS_2 the rate of II, the dissociation of the complex, is negligible compared with the rate of III, the reaction with I_2, the fraction may be neglected in comparison to I, and we will thus arrive at the result HOFMAN-BANG obtained: the rate of N_2–formation is proportional to catalyst and azide concentration, and independent of iodine. If on the other hand k_{II} large compared with k_{III}, then I may be neglected and we arrive at

$$s = \frac{k_I \cdot k_{III}}{k_{II}} \cdot [RSSR] \cdot [N_3^-] \cdot [I_2]$$

which is identical with equation (5).

In this connection it is worth mentioning that HOFMAN-BANG (5) has been able to increase the rate of N_2–evolution by incubating carbon disulfide and azide for a long time, while our similar experiments

with cystine (2.4) did not show any increase, but rather a small decrease. It is thus possible to accumulate the intermediate carbon disulfide-azide complex, while no accumulation of a cystineazide complex could be demonstrated. This indicates directly the difference in the rate of dissociation of the complexes assumed in the above considerations.

Unintentionally we have got some results on the cystine oxidation by iodine which can be compared with the results of SHINOHARA and KILPATRICK (10). These authors determined the rate of cystine oxidation from the rate of iodine consumption. This procedure necessitates working with low iodine concentrations, and accordingly, with very long reaction times.

Although 5 moles of iodine are consumed by each mole of cystine, they found the reaction to be of second order, the rate being proportional to the concentrations of iodine and cystine. They concluded "that the rate-determining step in the oxidation is the reaction between a molecule of cystine and a molecule of iodine (or substances whose concentrations are proportional to their concentrations)". It is not easy to compare their experiments with ours, but experiment *F* (fig. 1) in their paper comes so close to ours with regard to iodide and salt concentrations that it is possible to introduce rough numerical corrections for the small differences in the experimental conditions. It can thus be estimated that SHINOHARA and KILPATRICK if working at the same pH, total salt and iodide concentration as we, would have found a second order velocity constant of at least 0.70.

Since we kept the iodine concentration constant, we found the reaction to be of first order, but our first order velocity constant $k \bullet (I_2)$, when divided by (I_2), should be directly comparable to the second order constant of SHINOHARA and KILPATRICK. The average value of *k* in our experiments was 0.128 (table 4). The cystine oxida- tion thus seems to proceed at a considerably lower rate in the presence of azide. The reason for this remains obscure.

Our results discussed above thus establish a difference in the mechanism of the iodine-azide reaction, when this is catalyzed by f. inst. CS_2 and when catalyzed by cystine. Very little information has been obtained with respect to the more detailed aspects of the kinetics of the iodine-azide reaction, but since this was of no importance for our later work no experiments were done in the attempt to solve this problem.

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SUMMARY

The rate of N_2–evolution in the iodine azide reaction has been found to be proportional to the concentrations of iodine, azide ions and cystine. The cystine oxidation by iodine was found to proceed at a considerably lower rate with azide present than without azide.

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